Florida Citrus Advanced Technology Program

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
● *Quarterly Report* ○ *Annual Report* ○ *Final*	Psyllid	

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

HLB Pathogen Confirmed in Psyllid Digestive System (Filter Chamber and Alimentary Canal) and Salivary Glands

TITLE and CONTACT INFORMATION

Proposal Title Pathogen-Vector Relations between Asian Citrus Psyllid and Liberibacter asiaticus

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Organization USDA-ARS	*Total Direct Funds (current year)* $148,207

REPORT UPDATE (650 words; provide details about your headline)

1: Testing various techniques for localization of Liberibacter asiaticus (Las) in internal organs of Asian citrus psyllid (ACP). In order to study the cellular interactions of Las in its psyllid vector, the following three techniques have been tested for localization of this bacterium in dissected organs of ACP and in leaf sections and extracts of HLB-diseased plants:
A. Immunofluorescence confocal laser scanning microscopy using polyclonal and monoclonal antibodies. Two polyclonal antibodies (A and B), a mixture of both (C), and a mixture of three monoclonal antibodies prepared against Las membrane proteins have been tested at various dilutions (1/20 to 1/400) and incubation times (3-24 hrs). However, no specific fluorescence associated with Las bacterium was detected in insect organs or leaf sections.
B. Fluorescence In situ hybridization (FISH) based on oligonucleotide primers of the Las bacterium. Two oligonucleotide primers based on the following sequences of Las were tagged with Alexa Fluor 488: Primer 1 (20 bases): TCGAGCGCGTATGCAATACG; Primer 2 (30 bases): TCCCTATAAAGTACCCAACATCTAGGTAAA. So far, we tested primer 1 above, using several FISH protocols on dissected organs of ACP and on leaf sections and extracts from healthy and HLB-diseased citrus plants. Carnoy's fixative coupled with TBS washing produced the best results. Green fluorescence, indicating Las, was detected in the filter chamber and midgut of field-collected ACP, but not in healthy controls form the lab colony. It was also detected in leaf sections and plant extracts from HLB-diseased plants but not in those from healthy plants. We will replicate these experiments further to confirm these results and to refine the FISH procedure if required. We will also test the second primer (above) in future experiments.
C. Quantitative RT-PCR of dissected insect organs from individual ACP. Currently, RT-PCR has been the most reliable method for detecting Las in diseased plants and in vector ACP. However, to our knowledge, RT-PCR has only been applied to whole insects but not to insect organs of ACP. Thus, we investigated whether RT-PCR can be used to detect Las in the salivary glands and alimentary canals of individual ACP adults. In two experiments, RT-PCR detected Las in 7/24 (29%) of the salivary glands, 6/24 (25%) of the alimentary canals and 7/24 (29%) of the rest of the body of field collected insects, compared to 0/8 similar organs from each healthy control insect. Thus, we plan to use RT-PCR, FISH and other techniques to study the route, replication and transmission barriers of Las in its psyllid vector at the cellular, tissue and organ levels.
2. Testing the ability of field collected ACP to infect citrus species/varieties. Forty young citrus trees (Duncan grapefruit) growing in pots were each infested by 10 field-collected adults. After one week, these psyllids were removed and tested by RT-PCR. Three plants were dropped from the study because none of the psyllids recovered from them tested positive for Las. Among the other 37 plants, the percentage of psyllids that tested positive from each plant ranged from 12.5 to 71.4% with an overall mean of 36.5%. The plants were tested monthly for Las using RT-PCR. Eleven percent of the plants tested positive within one month. The percentage of plants testing positive for the pathogen increased to 30, 41, 43, and 46% at 2, 3, 4, and 5 months, and 57% of the plants tested positive during the 9th and 10th months after infestation. The experiment is being continued, and two similar studies were initiated with other citrus varieties.
3. Establishing new vector-efficient ACP colonies. Our ACP lab colony, established during 2000, has been maintained without adding wild types. Recently, tests indicate that insects from this lab colony acquire and transmit the HLB pathogen at much lower rates than field-collected ACP. New disease-free colonies have been established from field-collected ACP, and the ability of these to acquire and transmit HLB is being studied.